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(54) [Title of Invention]
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Abstract

(57)[(57) [Abstract]

[[Objective]

体液可溶性E・・・・・量・測定す ・・・・・・ ・・・提供性腫瘍・検出す ・方法・及・キ ・・ Method of detecting malignant tumor by measuring body fluid solubility Ecadherin quantity. And kit is offered.

[[Constitution]

体液中・体液可溶性 E ・・・・・量・測定・・ そ 法・ detection method ・ of malignant tumor which measures body fluid solubility Ecadherin quantity in body fluid,compares value with healthy value

体液可溶性 E ・・・・・・反応性・有す ・抗体 ・含有す ・・上記検出方法・実施す ・た め 性腫瘍検出用キ malignant tumor assay kit ・ in order contains antibody which possesses reactivity in the body fluid solubility Ecadherin, to execute above-mentioned detection method

体液・例・・血清・血漿・及・尿・・・・ There is a blood serum ・ blood plasma ・ and a urine as example of body fluid.

[[Effect(s)]

悪性腫瘍・検出・迅速・簡便・行・・・・・き ・・ It detects malignant tumor quickly and simply, it is possible .

Claims

[[Claim(s)]

[1・ [Claim 1]

体液中・体液可溶性 E ・・・・・量・測定・・ そ 性腫瘍・検出方法・ body fluid solubility Ecadherin quantity in body fluid is measured, value is comparedwith healthy value detection method ・ of malignant tumor which is made feature

[2・ [Claim 2]

体液可溶性 E ・・・・・・反応性・有す ・抗体 ・用・・体液可溶性E ・・・・・量・測定す ・ 請求項 1 記載・方法・ method ・ which is stated in Claim 1 which measures body fluid solubility Ecadherin quantity making use of antibody which possesses reactivity in the body fluid solubility Ecadherin

[3・ [Claim 3]

体液・・・血清・血漿・又・尿・用・・請求項 1 又・2 記載・方法・ method ・ which is stated in Claim 1 or 2 which uses blood serum ・ blood plasma ・ or the urine as body fluid

[4・ [Claim 4]

体液中・体液可溶性 E ・・・・・量・測定・・ そ 癌・検出・行・た め 溶性 E ・・・・・・反応性・有す ・抗体・含有 ・す・・・・・特徴・す ・悪性腫瘍検出用キ body fluid solubility Ecadherin quantity in body fluid is measured, with kit in orderto detect malignant tumor value is compared with healthy value with, antibody which possesses reactivity in body fluid solubility Ecadherin is contained malignant tumor assay kit ・ which is made feature

JP1994324046A 1994-11-25

Specification

【

【0001・

【

・本発明・悪性腫瘍・検出方法及・検出用キ
・・関す ・・

【0002・

【

・・・・・・分子量約0 万前後・膜貫通・・・
・持・細胞膜表面分子・・・・繼-細胞間
・接着・関・・ 共存す ・Ca^{2+}・・・依存的・
機能・発揮す ・・・・質・・・・

現在・・・・・・・・・数・分子種・発見・
・・・・・そ ・最・古・単離同定・・た
分子種・・・E・・・・・N・・・・・P・・・・
・・・3 種・・・知・・・・・。

・・・・ 3 種・分子種・そ・・・同一分子種
同志・・・選択的・結合す ・慣・示す ・・
・知・・・・・・・・・同質結合機構・・・
・・ 族・特徴・1 ・・考・・・・・・・

・・・・・・・・・分子種・・特生命体・発
生・分化・過程・組織構築・働・重要・細胞
間接着分子・・・・・知・・・・・・神経組
織・心臓・胎盤・・・た生体内・特定・臓器
・そ・・・一定・分子種・主要・分布・・・
・・・多・・そ・・・・組織・形成・細胞接
着機能・発揮・・・・考・・・・・・M.・・
・・(M.Takeichi) ・・・・・・(Science)・第251
巻・第1451~1455 頁(1991)・・

・・・代表的 3 種・・・・・・・・・E・・・・
・・・・・・・・・・・・・・上皮細胞・上皮
組織・主要・発現す ・分子・・ 免疫組織染
色法・・・用・・様・・疾患・組織中・局在
・・・調・・・・・・・・Y.・・・・・
(Y.Shimoyama)・・キ・・・・・・・・・(Cancer
Research)・第49 巻・第2128~2133 頁(1989)・・

そ・・・検討・結果・・・特・悪性腫瘍組織
・・・・E ・・・・・発量・減少・・・ 現
象・数多・見出・・・・・・

・た 動物実験・・・・・・悪性腫瘍細胞株・
・・E ・・・・・発量・多・細胞間接着・強
・株・比・・E ・・・・・発量・少・・細胞
間接着・弱・株・方・悪性度・高・・高・確

[Description of the Invention]

[0001]

[Field of Industrial Application]

this invention regards detection method and assay kit of malignant tumor.

[0002]

[Prior Art]

cadherin with plasma membrane surface molecule which has membrane penetration domain approximately of molecular weight approximately 100,000, is protein which showsfunction in Ca^{2+}ion dependent which coexists in regard to glueing between the cell-cell.

To presently molecular type is discovered by cadherin many, 3 kinds of the Ecadherin •Ncadherin •Pcadherin are well known even among those to be oldest as molecular type which isolation and identification is done.

As for molecular type of these 3 kinds only respective same molecule kind the selectively shows property which is connected, it is known , these same material coupling mechanism are thought one of feature of cadherin family.

These cadherin molecular type are important intercellular adhesion molecule which with process of occurrenceand differentiation of especially living thing works in organization construction, it is known , in specific organ of inside the body such as nerve organization and heart and placenta respective fixed molecular type distribution has made principal, is many , It is thought that cell adhesion function is shown in formation of therespective organization, {M. bamboo I jp8 (M.Takeichi), Science (Science), second Vol.51 • 14th 51~1455 page (1991)}.

Among cadherin of these representative 3 kinds, as for Ecadherin with molecule whichis revealed principally in epithelial cell and epithelial tissue in human and mouse etc, localized in organization of various disease such as isinspected making use of immunohistological staining method etc, {Y. ti haze • (Y.Shimoyama) and others, Cancer Research (0008 - 5472, CNREA8) (Ca ncer research), Vol.49 • second 128~2133 page (1989)}.

phenomena which Ecadherin amount of expression has decreased from result of thoseexamination, in especially malignant tumor organization is many discovered.

In addition, regarding animal experiment, tendency which forms transfer lesion with probability where Ecadherin amount of expression to be less strain where intercellular adhesion is weakdegree of malignancy is higher intercellular

率・転移病巣・形成す 傾向・見出・・・・・U.H.・・・・・(U.H.Frixen)・・・・・・・・・・・・・・・・・・・・(Journal of Cell Biology)・第 113 巻・第173~185 頁(1991)・・

・・・・結果・・・細胞表面・・表示・・・・E・・・・・・・量・悪性腫瘍転移性能・悪性度・間・・・密接・関連・・・推測・・・・・・

[0003・

・た一方・・・本質的・膜貫通領域・分子内部・有す・・・・・・界面活性剤・・・含・溶媒中・・・・・・・可溶化す・性質・持・・・た 界面活性剤・含・・・溶媒中・・・・・・細胞抽出液・・・・・・・・・・・・・分子・細胞外領域・一部・極め・・・・・・・・・・切断・・易・・切・出・・た 分子量8 万前後・低分子・・・・・・解断片・界面活性剤・・・含・・・水系溶媒中・可溶化状態・存在・き・・・・既・知・・・・・・・M.J.・・・・・・(M.J.Wheelock)・・・・・・・・・・・・・・・・・・・・・・・・・・・・(Journal of Celluar Biochemistry)・第34巻・第187~202 頁(1987)・・

・・・・・・・・・・・・・・・・・生・・・・・・・分解物・・・・動物・・・生体液中・存在す・・・・・従来全 知・・・・・・・た・

・た・・・・・・・・従来・・・・解物・主要・構成分子・す・可溶性・・・・・・溶液濃度・測定・・含有量算出す・技術 存在・・・・た・・・起因す・・

[0004・

[解決・・・・す課題・

・た 古・・・解析・・き た 細胞上・E・・・・・発現量・悪性腫瘍・・・疾患・・関連・・・・・従来E・・・・・・検出技術・・・用・・・き た 抗体・ 組織化学的検出法・・・・・・・・・・・・・・・・・・・・・・・・・・法・免疫沈降法・・・方法・・・・・定性的方法・・・・・・・・・患者・・・・・・組織生検材料・採取・危険・伴・た・試薬・機器・準備・・・時間・・・・・・・・点・・・・・・実際 疾患・診断・・・臨床的応用・極め 難・・・・・・・た・

本発明・目的・体液可溶性E・・・・・・・簡便

adhesion is strong inside Ecadherin amount of expression of malignant tumor cell line to be many in comparison with strain where, is high seesand comes out and ・ ・ is {U.H. ・ jp9 habit ・ (U.H.Frixen) and others, journal * of * cell biology (Journal of Cell (0092 - 8674) biology), Vol.113 ・ 17th 3~185 page (1991)}.

From these results, it is presumed in quantity of Ecadherin whichis been indicator in in cell surface and transition talent of malignant tumor andbetween degree of malignancy that there is relation of intimate.

[0003]

Portion of extracellular domain of cadherin molecule it is easy to be cut off with thequite protease in addition on other hand, as for cadherin whichessentially possesses membrane spanning region in intramolecular section with property which solubilizing is done only in in solvent which includes theboundary surfactant etc, in addition, in cell extracted liquid etc in in solvent which does not include boundary surfactant, As for low molecular weight cadherin disassembly fragment approximately of molecular weight 8 0,000 which isquarried out in aqueous solvent which does not include boundary surfactant etc it can exist it is already known with solubilizing state, {M.J. fee lock (M.J. Wh eelock) and others, journal * of * cellular * biochemistry (Journal of Cell (0092 - 8674) uar Biochemistry (0006 - 2960, BICHAW)), Vol.34 ・ 18th 7~202 page (1987)}.

But, it was not completely informed until recently that cadherin lysate which it occurs by protease exists in human and animal or other organism liquid.

In addition, this until recently measures solution medium concentration of solubility cadherin whichdesignates cadherin lysate as principal constituent molecule, originates in also the technology which calculates content not existing.

[0004]

[Problems to be Solved by the Invention]

In addition, for a long time concerning relation between Ecadherin amount of expression and malignant tumor or other disease on cell which is analyzed with antibody which is useduntil recently as Ecadherin detection technology as for histochemical detection method and flow site ・ tri- and Western blot method and immunoprecipitation or other method which with qualitative method with thing, inrecovery of organization biopsy material from patient etc hazard accompanying, Those whose diagnosis or other clinical application of disease quite is difficult actuallyat or other point where time is required for reagent and thepreparation etc of equipment.

It is to offer method and kit where objective of this invention

・定量す ・方法確立・ 該方法・用・・悪性
・・提供す 腫瘍・検出す ・方法及・キ
・・・・

[0005・

[課題・解決す ・た め手段・

本発明・概説す・・・本発明・第1 ・発明・
悪性腫瘍・検出方法・関・・体液可溶性 ・・
・・・量・測定・・そ
・・特徴・す ・・

・た 本発明・第2 ・発明・悪性腫瘍検出用キ
・・・関・・体液中・体液可溶性・・・・・量
・測定・・そ
・悪性腫瘍・検出・行・た め
体液可溶性 E ・・・・・・反応性・有す ・抗体
・含有す ・・・・特徴・す ・・

[0006・

本発明者・・以上述・・・た 従来技術 問題
点・考慮・・体液可溶性E ・・・・・・溶液中
微量濃度・簡便・測定す ・方法確立・・そ
測定法・・・・及・動物・体液中・体液可溶
性E・・・・・一定量存在・・・・・・・見出
・・そ E ・・・・量宿主・疾
患・特・悪性腫瘍・・・・有意・上昇す 現
象・見出・・本発明完成す ・至・た ・

・た ・本発明・方法・用・・た 試薬類・・
・め 化・・・・簡便・測定・簡・・
た ・

[0007・

本発明・・・・測定対象・・・体液可溶性・
・・・・・・・可溶性・体液中存在す ・E・・
・・・・分解物・・・・非分解物・・ E ・・・
・・分子・由来す ・す ・・分子・含・・そ・
・・主・分子量8万前後・ E ・・・・・解物
・・・構成・・・

[0008・

本発明・・・・体液可溶性E ・・・・・・測定
方法・・・・例 ・体液可溶性 E ・・・・・・
反応性・有す ・抗体・用・・方法挙・・・
・・

該方法・例・・・・・・・・・・・・・・・・
(EIA)・・・・・・・・・・・・・・・・・・
・・・免疫比濁法・・・・・・凝集法・・体液可
溶性 E ・・・・・・測定・き ・方法・・何
・・・・・・操作・簡便・・・・・・・特・EIA
・望・・・・

body fluid solubility Ecadherin simply establishes method which quantification is done,detects malignant tumor making use of said method.

[0005]

[Means to Solve the Problems]

If this invention is outlined, first invention of this invention regards the detection method of malignant tumor, it measures body fluid solubility Ecadherin quantity, value it iscompared with healthy value makes feature.

In addition second invention of this invention regards malignant tumor assay kit , itmeasures body fluid solubility Ecadherin quantity in body fluid, with kit in order todetect malignant tumor value is compared with healthy value with ,antibody which possesses reactivity in body fluid solubility Ecadherin it is contained it makes feature.

[0006]

As for these inventors above problem of Prior Art which is expressed isconsidered, method which trace amount concentration in solution of body fluid solubility Ecadherin measures simply is established, body fluid solubility Ecadherin constant amount exists in the body fluid of human and animal with measurement method , phenomena which youdiscover , body fluid solubility Ecadherin quantity rises significantly in disease • especially malignant tumor of host index, this invention it reached to completion.

In addition, collecting reagent in order to use for method of the this invention, to kit it converted, made simpler measurement possible.

[0007]

Regarding to this invention, body fluid solubility Ecadherin which becomes measurement subject, includingall molecule which such as lysate in Ecadherin molecule or nondegradable ones of Ecadherin which with solubility exists in body fluid derive, as for those mainly configuration it is done by Ecadherin lysate approximately of molecular weight 8 0,000.

[0008]

You can list method which uses antibody which possesses reactivity in for example body fluid solubility Ecadherin as assay of body fluid solubility Ecadherin in this invention.

If enzyme immunoassay (EIA), it is a method which such as radioimmunoassay • fluoro-immunoassay • immunity turbidimetric method • latex coagulation method body fluid solubility Ecadherin canmeasure as example of said method, it is good anything, but theespecially EIA is desirable from fact that operation is simple.

【0009・

体液可溶性E・・・・・・反応性・有す ・抗体・・・・・・・・・・抗体又・・・・・・・・抗体・・・・・・・・・・た 抗体由来動物・・・・・・・・・・・・・一般・実験動物・・・用・・・・動物・・・・・・・使用・・・・・・抗原・対す ・特異的反応性・確実・・・大量製造・容易・・・・・・・・・由来・・・・・・抗体・使用す ・・・・特望・・・・

・・・由来・・・・・・ 抗体・例・・・・ HECD-1(宝酒造社製) ・SHE78-7(宝酒造社製) ・・・た 類品・・・・本発明者・・新た 作製・たSHE13-6 ・SHE3-10 ・挙・・・・・

・・・4種・・・・・・・抗体・E・・・・・分子上・互・・異・・部位・認識す ・・任意・組合・・EIA系・構築す ・・・・・き ・・

例・・・HECD-1 ・ SHE3-10 ・組合・・SHE78-7 ・SHE3-10 ・組合・・EIA系・構築す ・・・・・き ・・

・た SHE13-6・体液可溶性E・・・・・対・・強・反応性・示・・細胞上・非断片化 E ・・・・・・弱・反応性・・示・・・・

・た ・・ SHE13-6・使用す ・・・・・・・・・均一・体液試料中・体液可溶性E・・・・・・測定す ・・・・能・・・・

・・・・抗体・当業者・・・・公知・技術・・・適宜・断片化・標識化・固定化・修飾化・・・処理・施・・・・・・・・

【0010・

本発明・使用す ・体液・・・・・血清・血漿・尿・髄液・羊水・腹水・・・・液・・通常・体外診断医療・・・・採取・・・・試料す・・・含・・組織・細胞・溶媒・・・・・洗浄液・抽出液・含・・・・す ・・・特頻繁・用・・・採取・簡便・・・・・・・血清・尿・好・・・

【0011・

本発明者・・・体液可溶性E ・・・・・測定法・・・・・・・・・・EIA ・・体液可溶性E・・・・・・反応性・有す2種・異・・・・・・・・抗体・一方・・・・・・・・・・・

[0009]

It is good with whichever of polyclonal antibody or monoclonal antibody as antibody whichpossesses reactivity in body fluid solubility Ecadherin, in addition antibody derivative animal mouse • rat • rabbit etc generally it is possible to use insidewhichever of animal which is used as experimental animal, but specific reactivity for antigen being secure, uses mouse derivative monoclonal antibody from fact that large scale production is easy especially is desirable.

HEC D-1 (Takara Shuzo Co. Ltd. (DB 69-053-7063) supplied), SHE78-7 (Takara Shuzo Co. Ltd. (DB 69-053-7063) supplied) with other than commercial product which was said,you can list SHE1 3- 6 •SHE3- 10 which these inventors produces anew as example of the mouse derivative monoclonal antibody.

Because monoclonal antibody of these 4 kinds recognizes site where topof Ecadherin molecule differs mutually, EIA system can be constructed withcombination of option.

EIA system can be constructed with combination of combination, the SHE78-7 and SHE3- 10 of for example HEC D-1 and SHE3- 10.

In addition, SHE1 3- 6 it shows strong reactivity vis-a-vis body fluid solubility Ecadherin,only weak reactivity shows in non- fragmentation Ecadherin on cell.

Therefore, it depends on using SHE1 3- 6, from body fluid solubility Ecadherin in body fluid specimen is measured is possible in uniform.

As for these antibody fragmentation • labelling • fixation and decoration conversion or other treatment may be administered appropriately by known technology in person skilled in the art.

[0010]

As body fluid which is used for this invention, including specimen everythingwhich can recover in conventional outside the body diagnosis medical care such as blood serum • blood plasma • urine • spinal fluid • sheep water and spleen • phosphorus pas liquid , also washing liquid and extracted liquid include with such as organization and solvent of cell it does, but it is used by especially frequent and blood serum and urine are desirable from fact that recovery issimple.

[0011]

As for these inventors, sandwich type EIA, it succeeded in to adsorb into microplate or other solid phase,labelling doing other with peroxidase enzyme, establishing one side of monoclonal antibody where 2 kinds which possess reactivity

固相・吸着・他方・・・・キ・・・・酵素・標識・・両者・組合・・・・・・初め 確立す ・・・・確・た ・

in body fluid solubility Ecadherin differfor first time by union doing both as body fluid solubility Ecadherin measurement method.

・・体液可溶性E・・・・・測定IA 法・・数ng/ml ・・低・濃度・溶液中・体液可溶性 E ・・・・・・定量・能・・・・従来・・種・体液可溶性 E ・・・・・測定法・確・・・・・・

As for this body fluid solubility Ecadherin measurement EIA method, you call several ng/ml,quantification of body fluid solubility Ecadherin in solution of low concentration being possible,body fluid solubility Ecadherin measurement method of this kind is not reported until recently.

・た ・本発明者・・・体液可溶性 E ・・・・・測定 EIA ・用・・・健常・成人・血清・血漿・及・尿中・数ng/ml ・・数μg/ml・範囲・体液可溶性 E ・・・・・・検出・・・・・・見出・た ・

In addition, as for these inventors in blood serum * blood plasma * of healthy human adult and in the urine from several ng/ml body fluid solubility Ecadherin can be detected in range of the several ;mu g/ml making use of this body fluid solubility Ecadherin measurement EIA, youdiscovered .

従来・・・又・動物由来・・・種・体液中・体液可溶性 E ・・・・・存在す・・・事実・見出・・・・・・た ・

Until recently, fact that was not discovered body fluid solubility Ecadherin exists in body fluid of this kind of human or animal derived.

【0012・

[0012]

更・・本発明者・・・・・・発明・発展・・・数十名・疾患患者・体液中・体液可溶性 E ・・・・量・布・数十名・健常人体液中・体液可溶性 E ・・・・・量・布・比較・統計的・解析・・悪性腫瘍患者・・・・有意・そ・値・上昇す 現象・見出・・腫瘍 ・・・・・病気・診断・病状・把握・・・極め用・・・・・・見出・た ・

Furthermore, these inventors these inventions developing, compares distribution of body fluid solubility Ecadherin quantity in body fluid of disease patient of several tens name, and the distribution of body fluid solubility Ecadherin quantity in healthy person body fluid of several tens name analyzesstatistically, phenomena where value rises significantly in the malignant tumor patient index, Quite it is useful in diagnosis of disease and grasp etc of the disease condition as tumor marker, you discovered .

そ 試料・・・用・・・・・・き・・・示す 共・・更・採取・簡便・尿・体液試料・・・用・・・・可能・・・・・示・た ・

As and, blood serum you can use it shows , as body fluid specimen furthermore also it is possible to use urine whose recovery issimple as body fluid specimen it showed .

・・様・体液可溶性 E ・・・・・・簡便・体液中・存在す ・腫瘍 ・・・・・・用・た 例・過去・・・・

This way as for reported example which uses body fluid solubility Ecadherin as tumor marker whichexists in simple body fluid there is not a past.

【0013・

[0013]

・た ・本発明・方法・用・固相化抗体液・標識抗体液・標準品溶液・基質溶液・・・試薬類・・・め ・・・・・・・・・簡便・体液可溶性E・・・・・測定・行・・・・・き ・・

In addition, solidification antibody liquid and labelled antibody liquid which are used for method of this invention, collecting standard article solution * substrate solution or other reagent, compared to it measuressimply body fluid solubility Ecadherin by making kit, it is possible .

・・・キ 試薬・溶液状・・・・凍結乾燥品・・・・・

Furthermore, reagent which is included to kit is good evenwith solution state and it is good even with lyophilized product.

【0014・

[0014]

以上詳細・説明・た ・・・・本発明・・・従来不可能・・・た 体液中・体液可溶性・・・・・微量測定・可能・・・・血清尿・・・た一般臨床検査・用・・・・体液・使用・・従

As above explained in detail, until recently trace amount measurement of the body fluid solubility Ecadherin in body fluid which is a impossible became possible depending upon the this invention , using body fluid which is used for general

来存在・・・新規・腫瘍・・・・・・・・疾患・・・・・可能・・・た・

laboratory test such as the blood serum and urine monitor of disease became possible with the novel tumor marker which does not exist until recently.

【0015・

[0015]

【

[Working Example(s)]

本発明・実施例・・・・更・詳細・説明す・・・本発明・・・・実施例・限定・・・・・・・・・

this invention furthermore is explained in detail with Working Example, but the this invention is not something which is limited in these Working Example.

【0016・

[0016]

実施例 l

Working Example 1

(1)免疫原・調製

Manufacturing (1) immunogen

健常妊婦・・分・・時・採取・・た胎盤・・20mM ・・・生理緩衝食塩水(150mM NaCl・5mM $CaCl_2$ ・2mM NEM ・0.3mM PMSF ・0.2% NaN_3 ・含・・pH7.6)(以下 TBS ・略す)・・5回洗浄・付着・・・・血液 除去・た・

From healthy pregnant woman when giving birth placenta whichrecovers, 5 times was washed with 20 mM tris menses buff saline (150 mM NaCl ・5 mM Ca Cl_2 ・2 mM NEM ・0.3 mM PMSF ・0.2% NaN_3 are included, pH 7.6) (Below TBS you abbreviate.) and the blood which has deposited was removed.

洗浄・た胎盤 500g・TBS1・・・・共・・・・・・・中・・細片化・た・

placenta 500g which you washed with TBS1 liter flaking was done in the homogenizer.

充分・細片化・た後・高速遠心分離・・・抽出液上清・不溶物・・分離・た(2000g×30 分間)・

flaking after doing, extracted liquid supernatant and insoluble matter were separated into the satisfactory due to high speed centrifugal separation (2000 gX 3 0 min).

次・・・抗・E・・・・・・・・・・・・・・・抗体 HECD-1(宝酒造社製)10mg・・・・・・・活性化・・・・・・(・・・・・・社製)2g ・吸着・・・HECD-1 抗体固定化・・・・・・・調製・た・

Next, anti-human Ecadherin mouse monoclonal antibody HEC D-1 (Takara Shuzo Co. Ltd. (DB 69-053-7063) supplied) 10 mg cyanogen bromide activated Sepharose (Pharmacia make) adsorbinginto 2 g, it manufactured HEC D-1 antibody fixation Sepharose.

調製・た HECD-1 抗体・・・・・・・・・充・・・・TBS・・・・・・・洗浄・た・

Filling up HEC D-1 antibody gel which it manufactures in glass column, TBS being you washed column well.

引続き 先 調製・た胎盤抽出上清液・本・・・流・・そ 後 TBS1 ・・・・・十分・・・・洗浄・た・

Continuously, placenta extraction supernatant liquid which is manufactured firstwas let flow to this column, after that with TBS1 liter column waswashed in fully.

・・・・洗浄・た後・8M 尿素・含有す TBS・・抗体・・・・吸着・た 撮物質・溶出・た・

After washing column, antigen substance which adsorbs into antibody column with TBS which contains 8 Murea was liquated.

溶出・・た・・・・質溶液・透析・・・・・入・・・l 晩 4 deg C 条件下・・TBS5 ・・・中・透析・た・

Inserting protein solution which is liquated in dialysis tube, under overnight 4 deg Ccondition dialysis it did in TBS5 liter.

透析・・・完全・尿素・除去・た後・・・・溶出・・・・溶液・再・前記・ HECD-1 抗体固定化・・・・流・・前記 同様・操作・・・・抗体・・・・・抗原物質・溶出・た・

After removing urea completely with dialysis , this liquation protein solution was let flow to aforementioned HEC D-1 antibody fixation column again,antigen substance was liquated from antibody column with operation of beingsimilar to description above.

溶出液・・・・・・・・・(・・・・・・社製)・・・・・・質濃度1mg/ml程度・濃縮・ 2mM

It concentrated eluate in protein concentration 1 mg/ml extent with collodion pack (Sartorius supplied), dialysis itdid under

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$CaCl_2$ ・含・生理食塩水5・・・・対・・1晩 4 deg C条件下・・透析処理・た ・

overnight 4 deg Ccondition vis-a-vis physiological saline 5 liter which includes 2 mM Ca Cl_2.

得・・た・・・・質溶液・・抗・・E ・・・・・ ・・・・・・・・・抗体・・・HECD-1 ・強・ 反応す ・撰物質・主要・含・・・・ 骸抗原 物質・主・・・・・E ・・・・・・盤物・・・構 成・・・(以後・・・・・・・質抗原・ 標準 E ・・・・・・称す)・・

protein solution which it acquires includes antigen substance which reacts to the HEC D-1 which is a anti-human Ecadherin mouse monoclonal antibody strongly in principal, said antigen substance configuration isdone by human Ecadherin lysate mainly, (From now on, this protein antigen, is named standard Ecadherin.).

・・標準 E ・・・・・500 μg ・・・・・・ ・分注・・80 deg C 以下・条件・免疫原・・ ・・標準物質・・ 使用す 時・・凍結保存・ た ・

In 500;mu g at a time aliquot it did this standard Ecadherin in tube,when - using with condition of 80 deg C or less to as immunogen or the standard substance, freezing it retained.

【0017・

[0017]

(2)・・・・・・抗体・作製

Production of (2) monoclonal antibody

実施例 1-(1)・取得・・た標準 E ・・・・ 500 μg ・・・・・・・・・・・・・・・・(・・ ・・社製)・等量混合・ 6 週令 Balb/c ・・・5 匹(日本・・・社製)・腹腔内・等分・・投与・ た ・

Freund * Kong pre * jp7 * adjuvant (Difco make) with equivalent it mixed standard Ecadherin 500;mu g which isacquired with Working Example 1- (1), 6 -week-old Balb/cmouse 5 animals equal parts did in intraperitoneal of the(Clea Japan Inc. (DB 69-073-1062) supplied) and prescribed.

4 週間後・標準 E ・・・・ 500 μg ・・・・・ ・・・・TDM(・・社製)・等量混合・ 既免疫 ・・・5 匹・再・等分・・腹腔内投与・た ・

jp9 via ・・ bunt TDM (jp9 ・ supplied) with equivalent it mixed standard Ecadherin 500;mu g 4 weeks, later equal parts made again previous immune mouse 5 animals and the intraperitoneal administration did.

更・4 週間後後者・投与操作・再度同・・・5 匹・実施・た ・

Furthermore dosage operation of the latter of 4 weeks later wasexecuted for second time in same mouse 5 animals.

そ 3 日後・・そ ・・2 匹・・・・・脾 臓・摘出・・・・・・・・・・・・方法・ 従・・細胞融合・行・・・・・・・・細胞・ 作製・た ・

After 3 days, avulsion it did spleen from mouse of 2 animals among those, followed to method of Kellar and Milstein and and others did cell fusion, produced hybridoma cell.

標準 E ・・・・・・鼠・・使用・ ELISA 法 ・・標準 E ・・・・・・反応性・有す・・・ ・・・・抗体・産生す ・・・・・・・・選択 ・た ・

You used standard Ecadherin as antigen, you selected hybridoma which produces monoclonal antibody which possesses reactivity in standard Ecadherin with ELISA method .

す ・・・標準 E ・・・・・TBS 中・・希釈・ ・終濃度 10 μg/ml ・・・溶液 調製・ 96 穴・ ・・・・・・・(・・・社)・適量分注・・そ ・・4 deg C ・・24 時間静置・た ・・・・・・ 中・溶液・す・・廃棄・ 1%・・血清・・・・ ・・含・・ 酸緩衝生理食塩水(以下 BSA/PBS ・称す)・・・・・ 滴た ・37 deg C 条件下 ・・1 時間静置・た ・

Diluting namely, standard Ecadherin in TBS, it manufactured solution whichbecomes final concentration 10 ;mu g/ml, 96 -hole microplate suitable amount aliquot did in (jp10 ・・ corporation), after24 hours standing doing that way with 4 deg C, it abolished solution in the plate entirely, it filled up phosphate buffered saline (It names below BSA/PBS.) which includes 1% bovine blood serum albumin in plate, 1 hour standing did under 37 deg Ccondition.

そ 後・・・・・中・溶液・す・・廃棄・・・ 酸緩衝生理食塩水(以下 PBS ・称す)・・2 回 ・・・・洗浄・・上記・・・作製・・た す ・・・・・・・・・細胞・培養上清・そ・・・ ・・・・各穴・添加・た ・

After that, as though solution in plate is entirely abolished and twice plate is washed with phosphate buffered saline (It names below PBS.), it is a description above, the culture supernatant of all hybridoma cell which are produced was added to each hole of therespective plate.

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そ・・室温・・1時間・・・・静置・た後・PBS・・3回洗浄・た・	After standing doing 1 hour plate that way with room temperature, thrice youwashed with PBS.
次・・抗・・・IgG・・・キ・・・・標識抗体液(・・・・社製)・BSA/PBS・・適当・希釈・た溶液・・・・・各穴・適量・・添加・た・	solution which dilutes anti-mouse IgGperoxidase labelled antibody liquid (Cappel supplied) next suitably with the BSA/PBS at a time suitable amount was added to each hole of plate.
再・そ・・室温・・1時間・・・・静置・た後・PBS・・3回・・・・洗浄・残液・十分・取除・た・	Again, after standing doing 1 hour plate that way with room temperature., the thrice plate was washed with PBS, residual liquid was removed to fully.
ABTS基質液(・・・・社製)・使用説明書・従・・調製・洗浄終了後・・・・・各穴・適量・・添加・室温・・15分間静置・た・	Following ABTS substrate liquid (Amersham supplied) to use description, it manufactured, at a time suitable amount added to each hole of plate after washing ending, 15 min standing did with room temperature .
そ後・・・96穴・・・・・・・・・・・・・・・・(・・・社製)・・・・各穴・発色・波長410nm・吸光度・・算出・・・・・・・・・・比較・強・発色・得・・・・・・・・・・・・細胞株・選択・た・	After that, you applied you calculated as absorbance of wavelength 410 nm, youselected these 96 -hole microplate on plate reader (furo • supplied), coloration of each hole hybridoma cell line where strong coloration is acquired by comparison with the background.
・・様・・・標準E・・・・・・原・・強・抗原抗体反応・起・す抗体・産す・株細胞・・・19株・選別・た・	19 strain were sorted to this way, with standard Ecadherin as antigen as the strain cell which produces antibody which causes strong antibody-antigen reaction.
・・・19株・・・・・・・・細胞・対・・2回・限界希釈・・・・・・・実施・再・上記ELISA法・・・・・・・・・・・行・最・適切・・・・・・・・細胞・・・・株2株・最終選択・・・・・細胞・産生す・・・・・・・抗体・そ・・・SHE13-6・SHE3-10・命名・た・	It executed limiting dilution cloning of twice vis-a-vis hybridoma cell of these 19 strain, did screening again with above-mentioned ELISA method , final selected most appropriate hybridoma cell clone strain 2 strain, monoclonal antibody which these cell produce SHE1 3- 6 •SHE3- 10 it designated respectively.
SHE13-6・SHE3-10・・HECD-1・SHE78-7・同様・E・・・・・・産す・・・既・知・・・・・・・皮膚・・細胞A431表面・強・反応す・・・間接酵素抗体染色法・・確認・た・	In same way as HEC D-1 •SHE78-7 Ecadherin is produced strongly reacts to human skin cancer cell A4 31 surface which is already informed verified SHE1 3- 6 •SHE3- 10, by indirect enzyme antibody dye method .
・・・・・・・A431細胞表面・生理食塩水等・十分・洗浄・残留・・・・可溶性・・・・・・細胞表層・・除去・た後・・・・・抗体・・細胞表面E・・・・・・反応・間接酵素抗体染色法・・調査・た・・・SHE13-6・A431細胞表面・・弱・反応・・示・・・・・確め・・た・	But, you wash A4 31 cell surface in fully, with such as physiological saline after removing solubility Ecadherin which has remained from cell surface layer when reaction to cell surface Ecadherin of these antibody was investigated with indirect enzyme antibody dye method , only weakreaction you show SHE1 3- 6 in A4 31 cell surface , it was verified .
【0018・	[0018]
そ SHE13-6・SHE3-10・HECD-1・SHE78-7・同様・E・・・・・・産・・N・・・・・・産す・・・知・・・・・HeLa細胞表面・全・反応・・・・・・・・同様・間接酵素抗体染色法・・確認・た・	And, SHE1 3- 6 •SHE3- 10 did not produce Ecadherin in same way as HEC D-1 •SHE78-7 and Ncadherin is produced completely does not react to HeLacell surface whichis informed verified by after all similar indirect enzyme antibody dye method .
更・SHE13-6・SHE3-10・標準E・・・・・・適当量SDS・・・・・・・・・・・電気泳動法	Furthermore, SHE1 3- 6 •SHE3- 10 separated reacts to protein of molecular weight approximately 80,000 verified

・・・分離・・・・・・・・・膜電気的・転写・た物・用・た・・・・・・・・法・・・・HECD-1・SHE78-7・同様・分子量約 8 万・・・・・質・反応す ・・・確認・た ・

【0019・

・た ・・・・族・共通す ・慣・・・・・・・・・親・・・培養細胞・・・・・・・・・・・・・・・質分解酵素・・・・分散・・・際・・2~10mM ・・・・・・・・共存下・・・・・・・分解・・・そ ・・・分子・形・保・・残存す ・・2~10mM EDTA 共存下・・・・・・・・・・・不在条件下・・・・・・・・分解・・・・・・分子・消失す ・・・現象・確認・・・・・M.・・・ (M.Takeichi) ・・・・・・・・・・・・・・・・・・・(Developmental Biology)・第87 巻・第340~350 頁(1981)・・

SHE13-6 ・・10mM $CaCl_2$ ・含・ 0.02%・・・・溶液・・ ・社製)処理 A431 細胞抽出液中・分子量約 10 万・ E ・・・r・弱・反応性・示・ 10mM EDTA ・含・ 0.02%・・・・溶液(・・ ・社製)処理 A431 細胞抽出液中・・・・・物・・反応・・・・・・SDS・・・・・・・・・・電気泳動法及・・・・・・・・・法・・・確認・た ・

【0020・

SHE3-10 ・ ・HECD-1・SHE78-7 ・同様・10mM $CaCl_2$ ・含・ 0.02%・・・・溶液処理 A431 細胞抽出液中・分子量約 10 万・ E ・・・・・強・反応性・示・ 10mM EDTA ・含・ 0.02%・・・・溶液処理 A431 細胞抽出液中・・・・・物・・反応・・・・・・SDS ・・・・・・・・・・・電気泳動法及・・・・・・・・・法・・確認・た ・

・た SHE3-10 ・分子量約 8 万・・・胎盤由来可溶性 E・・・・・対・・ SHE13-6 ・同等・反応性・有す ・・・ELISA 法・・ ・確認・た ・

更・・SHE3-10 ・そ 表面・十分・洗浄・た A431 細胞表面・強・反応性・示・ SHE13-6 ・弱・反応性・示す ・・・・・・・・・・・・・・法・・ ・確認・た ・

【0021・

・・・・結果・・・・SHE13-6・SHE3-10 ・・HECD-1・SHE78-7 ・同様・標準 E ・・・・・・反応性・有す ー・・・・・・・・・抗体・・・・・確証・得た ・

該 SHE13-6 産生・・・・・・・・・Hybridoma SHE13-6 ・表示・命名・ ・工業技術院生命工

standard Ecadherin due to suitable amount SD Spolyacrylamide-gel electrophoresis , insame way as HEC D-1 ・SHE78-7 by western blot method which uses those which arecopied to electrical in nitrocellulose film.

[0019]

In addition, occasion where cultured cell etc which reveals cadherin as property which is in common to cadherin family, is dispersed with the trypsin (EC 3.4.21.4) or other protease , under 2 - 10 mM calcium ion coexisting cadherin is notdisassembled and maintains shape of that way molecule andremains, but Under 2 - 10 mM EDTA coexisting under or other calcium ion absent condition cadherin is disassembled and phenomena that is verified, molecule disappears,{M. bamboo I jp8 (M.Takeichi) and others, ・ ・ rope mentha jp11 * biology (development al biology), 8 th 7 volumes,third 40~350 page (1981)}.

SHE1 3- 6, it showed reactivity which is weak in Ecadherin of molecular weight approximately 100,000 in 0.02% trypsin (EC 3.4.21.4) solution (furo ・ supplied) process A 4 31 cell extracted liquid which includes10 mM Ca Cl_2 it does not react , by SD Spolyacrylamide-gel electrophoresis or western blot method itverified even in what ones in 0.02% trypsin (EC 3.4.21.4) solution (furo ・ supplied) process A 4 31 cell extracted liquid whichincludes 10 mM EDTA.

[0020]

SHE3- 10, it showed reactivity which is strong in Ecadherin of molecular weight approximately 100,000 in 0.02% trypsin (EC 3.4.21.4) solution treatment A4 31 cell extracted liquid which includes 10 mM Ca Cl_2 in same way as HEC D-1 ・ SHE78-7 it does not react , by SD Spolyacrylamide-gel electrophoresis or western blot method it verified even in what ones in 0.02% trypsin (EC 3.4.21.4) solution treatment A4 31 cell extracted liquid which includes 10 mM EDTA.

In addition, SHE3- 10 it possesses SHE1 3- 6 and identical reactivity vis-a-vis human placenta derivative solubility Ecadherin of molecular weight approximately 80,000, youverified by ELISA method .

Furthermore, SHE3- 10 shows reactivity which is strong in A4 31 cell surface which washed surface in fully SHE1 3- 6 shows weak reactivity you verified by ・ ・ flow site ・ tri- method.

[0021]

From these results, SHE1 3- 6 ・SHE3- 10 when it is a mouse monoclonal antibody which possesses the reactivity in same way as HEC D-1 ・SHE78-7 in standard Ecadherin, acquired conclusiveevidence.

said SHE1 3- 6 production hybridoma Hybridoma SHE1 3- 6 and it indicated, designated, the deposit did in Agency of

学工業技術研究所・寄託・た(FERM P-13389)・

・た 該 SHE3-10 産生・・・・・・・・・・ Hybridoma SHE3-10 ・表示・命名・工業技術院生命工学工業技術研究所・寄託・た(FERM P-14144)・

Industrial Science and Technology National Institute of Bioscience and Human-Technology (FERM P-13389).

In addition, said SHE3- 10 production hybridoma, Hybridoma SHE3- 10 and it indicated,designated deposit did in Agency of Industrial Science and Technology National Institute of Bioscience and Human-Technology (FERM P-14144).

【0022・

[0022]

(3)・・・・・・・抗体・特異性・確認 Hybridoma SHE13-6 (FERM P-13389)・・・・・・(和光純薬社製)・・・・・め投与・た Balb/c・・・10 週令(日本・・・社製)腹腔内・大量・増殖・・・投与後約 10 日経過・た時点・・・・・腹水・採集・硫安塩析法又・・・交換・・・・・・・・・一般・抗体精製・・用・・・・方法・・精製・行・精製 SHE13-6 ・得た ・

Verification Hybridoma SHE1 3- 6 (FERM P-13389) of specificity of (3) monoclonal antibody, with Balb/cmouse 10 week (Clea Japan Inc. (DB 69-073-1062) supplied) intraperitoneal which prescribes pre stain (Wako Pure Chemical Industries Ltd. (DB 69-059-8875) supplied) beforehandmultiplying in large scale, after prescribing with time point whichapproximately 10 days' passage is done you collected spleen from mouse,such as ammonium sulfate salting-out is well used for antibody purification generally or ion exchange chromatography yourefined, with method which acquired refining SHE1 3- 6.

SHE13-6 ・過・・素酸法・・・・・・キ・・・・(・・・・・・・・・・・社製)・・標識・た ・

SHE1 3- 6 labelling was done with peroxidase (Boehringer Mannheim supplied) with periodic acid method.

標識 E・・・・・吸着・た96穴・・・・・・・・固相・・・ SHE3-10 ・SHE78-7・又・HECD-1 ・吸着抗原・反応・・・・・・・・・・そ 依存的・前述・酵素標識 SHE13-6 ・吸着抗原・・結合・阻害・・・・・・・・ ELISA 法・・調査・た ・・両者・抗体間・・全・そ 競合阻害性・見出・・・・・た ・

Whether or not connection to adsorption antigen of theaforementioned enzyme labelling SHE1 3- 6 inhibition designates SHE3- 10 ・SHE78-7 ・ or HEC D-1 asadsorption antigen in dose dependent it reacts by , labelling Ecadherin 96-hole microplate where it adsorbs as solid phase, when you investigated with the ELISA method , completely that kind of competitive inhibition characteristic did not discoverbetween antibody of both.

す・・・SHE13-6 ・・他・3 種・抗体・す ・・・SHE3-10 ・SHE78-7・及・HECD-1 ・標準 E・・・・・摂・・結合・競合・・・・た ・・E・・・・・分子上 SHE3-10 ・SHE78-7・及・HECD-1 ・認識部位・・異・・部位・認識す ・・・確認・た ・

namely, SHE1 3- 6 did not compete to connection to standard Ecadherin antigen of antibody ・ namely SHE3- 10 ・ SHE78-7 ・ or HEC D-1 of other 3 kinds, therefore the SHE3-10 ・SHE78-7 ・ on Ecadherin molecule and recognition site of HEC D-1 site which differsis recognized verified .

Hybridoma SHE3-10 (FERM P-14144)・・調製・た精製 SHE3-10・及・前出・SHE78-7・・・・そ・・・同様・検討・行・・・・・自身・除・他・3 種・・・・・・・抗体・抗原結合・関・・競合・・・・・確認・た ・

It did respective similar examination Hybridoma SHE3- 10 (FERM P-14144) from concerningrefining SHE3- 10 ・ , and depicted above SHE78-7 which are manufactured in each caseit does not compete it verified in regard to monoclonal antibody and antigen connection of other 3 kinds which exclude itself.

・・・ SHE13-6 ・SHE3-10 ・SHE78-7 ・及・HECD-1 ・・・・・E・・・・・分子上異・・部位・認識す ・・・確認・た ・

Depending, you recognize site where in each case top of the Ecadherin molecule differs you verified SHE1 3- 6 ・SHE3-10 ・SHE78-7 ・ and HEC D-1.

【0023・

[0023]

(4)・・・・・・ EIA 系・構築

Construction of (4) sandwich EIA system

HECD-1 ・SHE13-6 ・そ・・・固相化抗体・酵

sandwich EIA was constructed with HEC D-1 and SHE1 3- 6

素標識抗体・・・・・・・・・EIA・構築・た・

as solidification antibody・enzyme labelled antibody respectively.

・・PBS・・終濃度 1mg/ml・HECD-1・含・溶液・調製・・・・化・・・・・終濃度 0.1%・・・・・添加・固相抗体液・調製す・・

First solution which includes HEC D-1 of final concentration 1 mg/ml with PBS is manufactured, sodium azide is added in order to become final concentration 0.1%, the solid phase antibody liquid is manufactured.

該固相抗体液・・・・・・・・・・・・・(0.1%・・化・・・・・・含・PBS)・・100 倍・希釈・た終濃度 10μg/ml・抗体溶液・96 穴・・・・・・・・・(・・・・・・・・・F-16・・・・社)・各穴・200μl・・添加・密封・・4 deg C・24 時間静置す・・

said solid phase antibody liquid 96 -hole microplate it adds 200;mu l at a time antibody solution of final concentration 10 ;mu g/ml which with coating buffer (PBS which includes 0.1% sodium azide) is diluted in 100 times, toeach hole of (module plate F-16・jp10・・ corporation), seals up and 24 hours standing does with 4 deg C.

次・・・・・・・溶液・捨・・・・キ・液(0.1%・・化・・・・・含・BSA/PBS)・・・・・各穴・200μl・・添加・密封・・37 deg C・1 時間静置す・・

Next, you throw away solution from plate, add 200;mu l at atime blocking liquid (BSA/PBS which includes 0.1% sodium azide) to each hole of plate, seal up and 1 hour standing dowith 37 deg C.

・・・・・PBS・・2 回洗浄・・測定試料又・標準 E・・・・・・800・400・200・100・50・0ng/ml 含有す 標準品溶液・・・・・・各穴・100μl・・添加す・・

plate twice is washed with PBS, measurement sample or standard Ecadherin 800, 400 and 20 0, 1 00, 50 and 0 ng/ml standard article solution which is containedis added 100;mu l at a time to each hole of plate.

そ・・室温・・1 時間静置・た後・PBS・・・・・各穴・3 回洗浄・・・・キ・・・・標識・たSHE13-6・適度・BSA/PBS・・希釈・・・・・各穴・100μl・・添加す・・

That way, with room temperature 1 hour standing after doing, thrice you wash the plate each hole with PBS, you dilute SHE1 3- 6 which peroxidase labelling is done moderately with BSA/PBS and add 100;mu l at a time toeach hole of plate.

更・室温・・そ・・1 時間静置・た後・・PBS・・・・・各穴・3 回洗浄す・・

Furthermore after 1 hour standing doing that way with room temperature , plate each hole thrice is washed with PBS.

・・・・・各穴・・溶液・・取除き・・・・・・・・・・・2 塩酸塩錠(・・・社)1 錠・基質溶解液(0.01%・過酸化水素・含・・・・酸緩衝液・pH5.0)10ml・溶解・・(終濃度 1mg/ml)・該基質溶液・・・・・・各穴・100μl・・添加・・そ・・15 分間静置す・・

It removes solution well than each hole of plate, ortho phenylenediamine dihydrochloride pill (Sigma Chemical Co.) substrate dissolved liquid (citric acid buffer・pH 5.0 which includes 0.01% hydrogen peroxide) melts 1 pill in 10 ml and (final concentration 1 mg/ml), said matrix solution adds 100;mu l at a time to each hole of plate, 15 min standing does that way.

次・1N 硫酸溶液・・・・・・各穴・100μl・・添加・酵素・・・発色反応・停止・・速・・96 穴・・・・・各穴・発色・・・・・・・・・(・・・社製)・・波長 492nm・吸光計測・・・定量す・・

It adds 100;mu l at a time 1 Nsulfuric acid solution to each hole of plate nextand stops coloration reaction with enzyme , 96 -hole colors each hole of theplate rapidly with plate reader (furo・supplied) with light absorption measurement of the wavelength 492 nm quantification.

標準 E・・・・0~800ng/ml・標準品溶液・吸光度・表示濃度・・・・検量線・作製・各測定試料液・吸光度・・検量線・用・・・体液可溶性 E・・・・濃度・算定す・・

measuring line is produced with with absorbance and indicator concentration of standard article solution of standard Ecadherin 0~800 ng/ml, body fluid solubility Ecadherin concentration is computed making use of measuring line from absorbance of each measurement sample liquid.

代表的・検量線・図 1・示・た・

representative measuring line was shown in Figure 1.

す・・・約0~800ng/ml・・低濃度域・測定可能・・・た・

It was a measurable with low concentration region, namely, approximately 0 - 800 ng/ml.

・・・SHE78-7・SHE3-10・そ・・・固相化抗体・酵素標識抗体・・た場合・・・上記・全 同様・・・・・・EIA・構築す ・・・・簡・・・た ・

Furthermore, sandwich EIA is constructed was possible completely in thesame way as description above even with when SHE78-7 and the SHE3- 10 are designated as solidification antibody・ enzyme labelled antibody respectively.

【0024・

[0024]

(5)体液試料・測定

Measurement of (5) body fluid specimen

悪性腫瘍患者血清51例・健常成人血清18例・悪性腫瘍患者尿 21 例・健常成人尿20 例・対象・・・HECD-1・SHE13-6・組合・・・・実施例1-(4)・記載・方法・・・体液可溶性・・・・濃度・測定・た ・

body fluid solubility Ecadherin concentration was measured due to method which with combination of the HEC D-1 and SHE1 3- 6 is stated in Working Example 1- (4) with malignant tumor patient blood serum 51 example,healthy adult blood serum 18 example, malignant tumor patient urine 21 example and healthy adult urine 20 example as object.

・・・・尿・随時尿・た め ・・・・・・・・・・・・・・・・・・・・・(和光純薬社製)キ・・・ 同時・測定・・尿中・体液可溶性・・・・・量・尿g・・・・・・当・・体液可溶性E ・・・・・値g/g・・・・・・)・・ 算出・た ・

urine among these on occasion because of urine, measured the creatine in urine simultaneously due to creatine * test * Wako (Wako Pure Chemical Industries Ltd. (DB 69-059-8875) supplied) kit ,calculated body fluid solubility Ecadherin quantity in urine body fluid solubility Ecadherin value per urine g* creatine (mg/g * creatine)as.

・た 試料・血清・尿・・・・ BSA/PBS ・・100 倍・希釈・測定・た ・

In addition, specimen with BSA/PBS diluted blood serum・ urine in each case in100 times and measured.

各血清中・体液可溶性E・・・・濃度・分布・図 2 ・各尿中・体液可溶性 E ・・・・・値・分布・図 3 ・示す ・

distribution of body fluid solubility Ecadherin concentration in each blood serum in Figure 2, distribution of body fluid solubility Ecadherin value in each urine is shown in Figure 3.

図 2 ・示す ・・・血清中・体液可溶性・・・・濃度・正常値上限・3μg/ml ・設定・た場合・悪性腫瘍陽性率・ 51%(26 例陽性/全 51 例中)・健常成人・全例陰性・・・た(0 例陽性/全 18 例中)・

As shown in Figure 2, as for malignant tumor positive ratio when normal value upper limit of body fluid solubility Ecadherin concentration in blood serum is set 3;mu g/ml 51% (In 26 example positive/ all 51 examples), as for healthy adult they were all theexample negative, (In 0 example positive/ all 18 examples).

・た 図 3 ・示す ・・・尿中・体液可溶性・・・・値 正常値上限・11.7mg/g・・・・・・認定・た場合・悪性腫瘍陽性率・ 57%(12 例陽性/全 21 例中)・健常成人・全例陰性・・・た(0 例陽性/全 20 例中)・

In addition, as shown in Figure 3, as for malignant tumor positive ratio when normal value upper limit of body fluid solubility Ecadherin value in urine is recognized 11.7 mg/g * creatine 57% (In 12 example positive/ all 21 examples), as for the healthy adult they were all example negative, (In 0 example positive/ all 20 examples).

・・・・・血清中・尿中・体液可溶性 ・・・・量 正常値・比較す ・・・・・・悪性腫瘍・検出・可能・・・た ・

This way detection of malignant tumor was possible by comparing body fluid solubility Ecadherin quantity in blood serum and in urine with normal value.

【0025・

[0025]

実施例 2

Working Example 2

キ 築

Construction of kit

表 1 ・示す ・・・体液可溶性 ・・・・・測定用 EIA キ 築・た ・

As shown in Table 1, body fluid solubility Ecadherin measurement EIA kit was constructed.

・・・・酵素標識抗体・精製・たSHE13-6 ・・・キ・・・・・酵素標識・ 核標識抗体・

Among these, SHE1 3- 6 which was refined enzyme labelling it did enzyme labelled antibody with peroxidase, with

終濃度 1mg/mlIgG 含・溶液・BSA/PBS ・・1000 倍・希釈・・・・・・・瓶・1 本・・き 11ml 封入・・そ ・・凍結乾燥・た・・・調製・た ・

BSA/PBS it diluted solution which said labelled antibody final concentration 1 mg/ml IgG is included in 1000 times, 11 ml it enclosed in glass vial bottle concerning 1, it manufactured those which lyophilizing are done that way.

検体希釈液・・0.1%・・化・・・・・・含・BSA/PBS ・0.22μm 口径・・・・・・・・・・・過滅菌・た・・・調製・た ・

test agent diluent BSA/PBS which includes 0.1% sodium azide manufactured those whichfiltration sterilization are done with membrane filter of 0.22;mu m aperture.

標準品・実施例 1-(1)・・調製・た標準 E・・・・・・0.1%・・化・・・・・含・BSA/PBS ・・希釈・800ng/ml 終濃度・・・溶液 作製・・・標準品溶液・・・・・・・瓶 1 本・1ml 封入・・そ ・・凍結乾燥・た・・・調製・た ・

standard Ecadherin which is manufactured with Working Example 1- (1) it diluted standard article with BSA/PBS which includes 0.1% sodium azide and it produced solution which becomes 800 ng/ml final concentration, 1 ml enclosed this standard article solution in glass vial bottle 1, itmanufactured those which lyophilizing are done that way.

そ 他・・実施例 1-(4)・記載・・・・使用・た ・

Other things used those which are stated in Working Example 1- (4).

[0026・

[0026]

[表 1・

[Table 1]

・内 容 物・ <contents>	容 量・ volume>		・本 < Book		数・ Number >
固相抗体液 solid phase antibody liquid	・μ・ 20;mu l	× X	・ 1	・・・・ vial	
・・・・・・・・・・・ coating buffer	・ml 11 ml	× X	・ 2	・・・・ vial	
・・・キ ・液 blocking liquid	・ml 11 ml	× X	・ 2	・・・・ vial	
酵素標識抗体 凍結乾燥品・ enzyme labelled antibody (lyophilized product)	・ml 用 11 ml	× X	・ 1	・・・・ vial	
標 準 品 凍結乾燥品・ standard article (lyophilized product)	・ml 用 1 ml	× X	・ 1	・・・・ vial	
検体希釈液 test agent diluent	・ml 11 ml	× X	・ 2	・・・・ vial	

・・・・・・・・・・・・錠 ortho phenylenediamine pill			・ 2	錠 pill		
基質溶解液 substrate dissolved liquid	・ ml 11 ml	× X	・ 2	・・・・ vial		
・穴・・・・・・・・ 96 -hole microplate			・ 1	枚		

【0027・

[0027]

【

[Effects of the Invention]

以上詳細・説明・た ・・・・本発明・・・・迅速・簡便・体液中・体液可溶性E・・・・・測定方法・確立・・・新規・悪性腫瘍・検出方法及・キ ・・た ・

As above explained in detail, being quick with this invention , body fluid solubility Ecadherin assay insimple body fluid was established, detection method and kit of novel malignant tumor were offered.

【図面・簡単・説明・

[Brief Explanation of the Drawing(s)]

【図・・

[Figure 1]

実施例 1-(4)・・・・・・・・・・EIA 系・使用す ・検量線・示す・・・・・・・

It is a graph which shows measuring line which is used with sandwich EIA system in Working Example 1- (4).

【図・・

[Figure 2]

本発明・方法・測定・た 血清中・体液可溶性E・・・・濃度・測定結果・示す図・・・・

It is a figure which shows measurement result of body fluid solubility Ecadherin concentration in blood serum whichwas measured with method of this invention.

【図・・

[Figure 3]

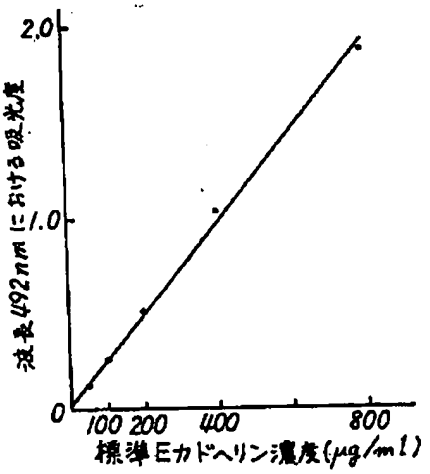
本発明・方法・測定・た 尿中・体液可溶性・・・・・値・測結果・示す図・・・・

It is a figure which shows measurement result of body fluid solubility Ecadherin value in urine whichwas measured with method of this invention.

Drawings

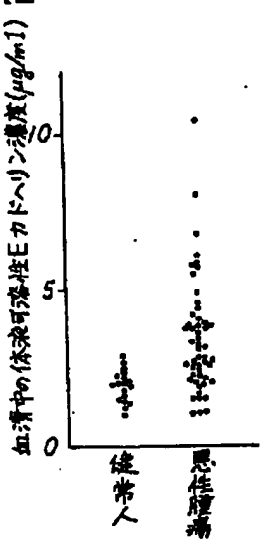
【図・・

[Figure 1]



【図・・

[Figure 2]



【図・・

[Figure 3]

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